Reproductive Biology of the Northern Quahaug, *Mercenaria mercenaria*, in Prince Edward Island.

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Landry, T., M. Hardy, M. Ouellette, N. G. MacNair and A. Boghen. 1999. Reproductive Biology of the northern quahaug, *Mercenaria mercenaria*, in Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 2287 : v +18p.

The northern quahaug, *Mercenaria mercenaria*, is an important species for both the commercial and recreational fisheries as well as for aquaculture purposes in Prince Edward Island. The management strategy of the quahaug resource is largely based on the minimum legal size of 50 mm. At the same time, there is a growing concern regarding the sustainability of the clam industry and hence, an evolving interest in stock enhancement. Effective brood-stock management, however, requires basic information about the animal's reproductive biology.

Sexual maturity, ovocyte size, gonado-somatic ratios and time of spawning were established for quahaugs sampled from two sites in West River, PEI This was achieved using histological methods and a determination of the animals' physiological condition indices. Findings revealed that the minimum size at sexual maturity was 25 mm and 30 mm (shell length) for males and females respectively. Furthermore, there was a positive correlation between ovocyte size and shell length. Seasonal variation coincided with spawning predictions based on conventional physiological condition indices. As well, the gonado-somatic contribution increased as a function of length. Both histological and condition index data support the likelihood that a major spawn occurs in mid-June. The study provides useful information on the reproductive biology of *M. mercenaria* and could contribute towards a reassessment of existing management and grow-out strategies.

RÉSUMÉ

Landry, T., M. Hardy, M. Ouellette, N. G. MacNair and A. Boghen. 1999. Reproductive Biology of the northern quahaug, *Mercenaria mercenaria*, in Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 2287 : v +18p.

Le quahaug nordique, *Mercenaria mercenaria*, est une espèce importante pour la pêche récréative et commerciale ainsi que l'aquaculture à l'Île-du-Prince-Édouard. En ce moment, la gestion du quahaug est basée sur une limite de taille minimale de 50 mm. Cependant, il commence à y avoir des inquiétudes en ce qui concerne la durabilité de cette ressource. Il y a donc un intérêt croissant pour trouver des techniques d'interventions possibles pour l'amélioration des stocks. Une gestion efficace des stocks nécessite plusieurs informations de base sur la reproduction.

Des techniques histologiques et la détermination de l'indice de condition physiologique ont été utilisées. La maturité sexuelle, la taille des ovocytes, le rapport gonado-somatique et la période de frai ont été déterminés à partir de quahaugs échantillonnés de deux sites à West River à l'Î.-P.-É. Les résultats ont démontré que le sexe dépend de la taille, la maturité sexuelle étant atteinte à 25 mm et 30 mm (longueur de coquille) pour les mâles et les femelles respectivement. De plus, la taille des ovocytes et le rapport gonado-somatique ont été corrélés positivement avec la longueur corporelle. Les analyses saisonnières d'ovocytes, de rapports gonado-somatique et du conditionnement ont révélé une concordance entre elles. Il semble y avoir une ponte principale à la fin du mois de juin. Toutes ces informations peuvent contribuer à des applications pratiques pour la gestion de *M. mercenaria*.

Introduction

The quahaug, *Mercenaria mercenaria* (Linnaeus, 1758), is a bivalve mollusc belonging to the Veneroida order and the Veneridae family. Its geographic distribution extends along the Atlantic coast from Florida to the Gulf of St. Laurence. The quahaug is usually identified by its thick, resistant and triangular shell, thus the common name "hard-shell clam". Annual growth rings on the exterior, a toothed margin and a purple stain on the inside mark the shell and are also identification characteristics. Its shell confers a good protection against most predators and notably the crab (Bricelj, 1993). Most predation occurs on juvenile clams that are smaller than 25 mm (anterior-posterior shell length), while clams that are larger, and whose shells are stronger, seem to be relatively predator-resistant (Ahn *et al.*, 1993).

M. mercenaria is a species with special importance in the Maritimes for commercial and recreational fishing as well as for aquaculture potential. In the past few years, the quantity of clam landings has been relatively stable. However, this stability is attributed to a greater exploitation of new stocks from contaminated areas that are transplanted elsewhere for depuration, rather than sustainable use of "clean" stocks. These fishing methods, however, are recognised as limited and temporary at best. This is why fishermen and government agencies are trying to develop enhancement strategies to help increase the longevity and productivity of this resource. Aside from the economic importance, the restoration of bivalve stocks can also contribute to the health of the ecosystem. Bivalves reduce the amount of suspended materials and link the benthic and pelagic production zones.

Most of the research on quahaugs has been done in the southern United States. For quahaugs, temperature is the principal factor influencing metabolism and gametogenisis (Dalton and Menzel, 1983; Manzi *et al.*, 1985). Therefore, because water temperature varies considerably according to latitude, aspects of the biology of the clam may also vary with climate. In Florida, for example, spawning can occur in January (Heffernan *et al.*, 1989), whereas clams in Atlantic Canada are under the ice at this time of year. Also, to reach the minimum legal size limit of 50 mm in Prince Edward Island, clams need at least 4 years in our climate (Landry *et al.*, 1993), while it may take only 2 years in Georgia (Bricelj, 1993). Proper management of natural stocks and the development of aquaculture require pertinent information with regards to the reproductive biology of this species.

West River is located near Charlottetown on the south shore of PEI (Figure 1). It is a relatively shallow estuary that empties into Hillsborough Bay, one of the most important areas in the province for commercial fisheries of oysters and quahaugs. It is estimated that this bay produces over 70% of the annual landings of quahaugs in PEI. Despite that, the natural stocks of quahaugs in West River are believed to have suffered important losses due to modifications of the sedimentation pattern caused by the construction of a causeway in the 1960s. The river was later reopened in the 1980 but the stocks have not yet fully recovered. The possibility of improving the stocks is therefore strongly supported by the local fishermen.

Many enhancement strategies presently exist to improve stocks for other molluscs like scallops and oysters. In oyster culture, substrate modification, through addition of broken shells, is used to increase larval settlement (Scarratt and Sephton, 1995). Collectors, of various types, are also employed for capturing spat that will be then transplanted to grow-out sites. These techniques are in widespread use for enhancement of the natural stocks as well as in aquaculture of the oyster in Canada (Scarratt and Sephton, 1995).

Effective techniques are not yet available for the quahaug. Collections of quahaug spat with techniques established for other species have been so far unsuccessful, therefore increased quahaug juvenile production has been limited to hatcheries. The cost associated with hatchery production is too high to be used for enhancement of natural stocks although it can be economically viable in a commercial context. There are substrate modification techniques in use for the quahaug fishery, but most of these are for predator control rather than for improving recruitment.

The present study is a first step towards having an understanding of the reproductive biology of the quahaug in PEI. The information gathered here will be used in developing enhancement strategies for natural and commercial stocks that are specific to the needs and climate of the Gulf of St. Lawrence.

Materials and methods

Study site

Quahaugs were purchased from the "PEI Shellfish Association" and transferred to West River in June 1998. Sizes ranged from 18 mm to 91 mm with a mode of about 50 mm. Two experimental plots where established at intertidal and subtidal water depths (low water mark and submerged 1 m at low tide) without predator control measures. Each plot had an area of 2 m^2 and quahaugs were distributed randomly in each at a density of approximately 250/m^2. Sampling began after a two-week acclimatisation period.

Sampling

Quahaugs were collected from each plot on a bi-monthly basis from June to August and on a monthly basis thereafter until October 1998. A sample of 120 clams was scheduled for every date. Clams were measured (anterior-posterior shell length) at West River with Mitutoyo electronic calipers. From each plot, 40 clams, equally distributed among the four size classes (Table 1), were taken for histological analysis (10 specimens each). In addition, another 20 clams of approximately 50 mm were taken to determine the condition index. Cumulatively, 437 quahaugs from 12 samples were used for histological analysis as well as 300 from 15 samples for condition indices.

Sampling was done with an apparatus using the Venturi principal in the intertidal plot and oyster tongs in the subtidal plot. A Venturi system consists of a water pump connected to a "T" shaped PVC pipe in such a way that water travelling through it creates a suction that can vacuum clams out of the substrate and collect them in a bag.

Specimens were kept cold and moist (salt water) in a cooler during transportation back to the laboratory. Thereafter they were refrigerated at 4°C until processing. Condition index and fixation for histology were always performed within 48 hours of sampling.

Condition index

The condition indices were calculated with the relationship between the dry weight of the organic material and the dry weight of the shell (Rainer and Mann, 1992).

C.I. = (tissue dry weight x 100/shell dry weight)

The shell was allowed to air-dry for four hours before being weighed. The tissue was dehydrated in a drying oven at 60°C for 24 hours then weighed. Afterwards, the dried tissue was gradually burned to ashes at 500°C for 24 hours so that all the organic matter would be removed. The weight of the organic matter is calculated by subtracting the ash weight from the dry tissue weight.

Histological analysis

Histological work and slide preparation followed the procedures described by Howard and Smith (1983). A standardised dorsal-ventral cross section of 5 mm was made in order to get the three major types of tissues: digestive gland, foot, and gonad. The digestive gland and an indentation at the base of the foot (always present) were used as reference points for sectioning.

Tissues were then fixed, 10% buffered formaldehyde, a minimum of five days before alcohol dehydration and infiltration of 60°C paraffin wax. Once hardened, 6 µm sections of the tissues were made using a rotary microtome and then transferred to microscope slides. The Eosin-Hematoxilin coloration was applied to the slides in order to bring out the differences in the three major tissues (Howard and Smith, 1983).

Sex determination

Sexes were determined by direct observations of the microscope slides. Spermatozoids and ovocytes are easily distinguished with a microscope. Their presence was noted in relation to shell length in order to determine the sex ratio and sexual maturity.

Ovocytes

The diameter of 15 ovocytes (eggs) was measured for each of the 183 females sampled using an ocular micrometer. The ovocytes were chosen randomly with five measurements from three different fields of view. However, there was a selection for ovocytes that were considered mature. The criteria used to establish maturity were a clear nucleus, a spherical form, and separation from the follicle (Davidson, 1998). Seasonal variation of ovocyte size was determined by making an average of at least three quahaugs (minimal total = 45 ovocytes) having a shell length between 50 and 60 mm.

Gonado-somatic ratio

The gonado-somatic ratio (G-S ratio) represents the relative percentage of gonad in the tissue section. The G-S ratio is determined by measuring the surface area of gonad dividing it by the total area of the section.

$$\text{G-S ratio} = \text{Area of gonad}/\text{Total area}$$

Slides were digitised using an optical scanner and then the areas were traced and calculated using SigmaScan software. All empty spaces (i.e. stomach cavity) in the tissue were excluded when tracing surfaces. Also, only complete tissue sections were used. The seasonal variation of the G-S ratio was determined by using clams with a shell length between 50 mm and 60 mm and making an average for a sampling date. By the same token, clams used in the determination of the G-S ratio versus shell length were taken from a brief time span in the beginning of the season.

Statistic analysis

The sex ratio was analysed with a Chi^2 test (Walker and Heffernan, 1995) with a null hypothesis being that there would be no significant difference between the sexes (1:1). Linear regressions for the ovocytes and the gonado-somatic ratio were analysed using an ANOVA regression test (Zar, 1996). In both types of tests, the signification level was set at 0.05.

Results

Condition index

The results of the condition indices are illustrated in Figure 2. On June 26, there is a difference between the two sites. The index for the subtidal plot drops slightly earlier than the intertidal plot. However, as a whole, there are few differences in the conditioning between the intertidal and the subtidal plots. Given the strong similarity, data were combined (without distinction between the two plots) for the following analysis. A decline in the condition index of 1.3 was observed between June 18 and July 29. The lack of three intertidal samples (Figure 2) from August 12 to October 26 was attributed to the destruction of the site by unauthorised fishing.

Sex determination

Sexes were determined in order to calculate the sex ratio and to identify the size of sexual maturity (Figure 3). Among the 437 slides prepared, the number of females, males, and unidentified were 183, 213 and 37 respectively. Clams that were deemed "unidentified" had no observed sexually differentiated cells. The Chi^2 analysis revealed that sex ratio was not significantly different from 1:1 for the size classes as a whole ($p>0.25$). However, the class of clams with a shell length smaller than 30 mm had significantly more males than females ($p<0.05$). Sexual maturity, the presence of sexually differentiated cells, began at 25 mm and 30 mm for males and females respectively.

Ovocytes

The average ovocyte diameter tended to increase with shell length (Figure 4) even though there is a large amount of variation between individuals. Its regression slope was statistically significant ($F_{0.05\,(1,\,28)}=13.32$, $p < 0.0025$) with an R^2 value of 0.094.

Seasonal ovocyte variation analysis showed that between June 18 and July 29 the average diameter varied around 60 µm with a maximum of 68 µm on June 29 (Figure 5). Following this

peak there was an important decline in the ovocyte diameter until September 25 where the ovocytes averaged 32 µm. In October, there was a tendency towards an increase in ovocyte diameter that was not significant.

Contribution

The gonado-somatic ratio was used in order to evaluate the relative contribution for gamete production. Seasonal variation of the G-S ratio is shown in Figure 6. The average G-S ratio reached a maximum of 38% on July 7 and then declined to 20% on September 25. There was also a non-significant tendency to increase in October.

There was a positive correlation in the G-S ratio as a function of shell length (Figure 7). Therefore, the percentage of gonad increases linearly with shell length. The linear regression slope for this relation was statistically significant ($F_{0.05\,(1,\,28)}$=101.65, $p < 0.0005$) and had a R^2 value of 0.784. This means that this relation explains 78.4% of the variation between the points.

Discussion

Sexuality

The sex ratio of 1:1 is consistent with those found by other researchers (Walker and Heffernan, 1995). Sexual maturity was strongly influenced by shell length (Figure 3). Other studies have shown that quahaugs are protandric hermaphrodites (Bricelj and Malouf, 1980). This was not fully confirmed because no hermaphrodites were observed during this project. Nonetheless, males did reach sexual maturity at a smaller size than females. According to Andersson (1994), protandry is an evolutionary strategy that allows small (or young) individuals to contribute effectively to reproduction. Smaller individuals (i.e. < 30 mm for quahaugs) theoretically would not have enough resources to produce a large number of costly ovocytes (energy and space) therefore they differentiate into males (Andersson, 1994). Thus, smaller individuals being male in their early lives would be favoured. Above 30 mm in shell length, the

sex ratio reaches equilibrium. In Figure 3, the 80 mm and 90 mm sizes had a frequency of 1 (100%) which is due to having only one animal in each of these classes.

Ovocytes

Bivalves produce a large number of eggs in order to compensate for the high juvenile mortality (Kraeuter *et al.*, 1982). Egg production in quahaugs is estimated in the order of several million per year (Kraeuter *et al.*, 1982; Bricelj and Malouf, 1980). However, ovocyte size is also very important because it has repercussions on growth and viability of juveniles. Kraeuter *et al.* (1982) observed that large eggs survived better than smaller ones and that even small differences in size had an impact. The size of the egg is directly related to the amount of energy reserves (fats and sugars). The present work showed that the egg diameter increased with shell length. This means that larger individuals tend to produce larger eggs that will have a better chance of survival.

Seasonal variation of the ovocytes also has important implications on fecundity. The line at the 50 µm mark in Figure 5 indicates the spawning size according to Bricelj and Malouf (1980). This means that unfertilised ova smaller than 50 µm were not found spawned naturally. Therefore, it is possible that the eggs (< 50 µm) observed in the sectioned tissue were too small to be spawned or less viable. A decline in ovocyte size at the end of July could mean that no spawning would take place or that ovocytes might have a smaller chance of survival. One possible cause of this decline is that the female is reabsorbing the eggs. Another hypothesis is that the average egg size would decease as the larger eggs are spawned. Observations suggest the latter even though it is not presently possible to be certain. The tendency for an increase in October is possibly explained by the rebuilding of the gonad for the next year. It is not likely that there would be another spawning event that year because when the water temperature drops bellow 10°C, metabolic activity stops (Rice, 1994). Bricelj and Malouf (1980) found that quahaugs would retain ripe ovocytes during the winter. Also, at the end of the summer there is a larger proportion of smaller eggs (Bricelj, 1979 cited in Kraeuter *et al.*, 1982). These reports correspond with seasonal variations observed in PEI.

Contribution

The seasonal study of the gonado-somatic ratio revealed gradual variations. The variation in the percentage of gonad is most likely due to the changes in the fullness of the gonad during spawning. The release of gametes would make the volume of the gonad decline. The increase in the G-S ratio in October also tends to indicate a rebuilding of the gonad at the end of the season.

Results of the percentage of gonad as a function of size differ from other studies. Several researchers have shown that the absolute amount of gonad increases with shell length (Peterson, 1986) or that the relative amount of gonad does not decrease with age (Peterson (1983), cited in Walker and Heffernan, 1996 and Peterson, 1986). However, our results suggest an increase in the percentage of gonad as a function of shell length, thus the larger the quahaug, the more its metabolism is dedicated towards reproduction. A 30 mm animal is composed of approximately 6% gonad, while an 80 mm quahaug is 60% gonad. Large clams are, therefore, very important in terms of reproductive biomass.

Spawning

The condition index is an indirect way of determining when the spawning event took place. It is a widely used technique for evaluating bivalve health and spawning activity (Rainer and Mann, 1992). A spawning event is identified by a relatively important drop (amount depends on the species) in the index in a certain time period. For quahaugs, variation of the index is relatively small compared to other species like the oyster. The greatest difference in the quahaug index measured this season was 1.3 points, while the index of oysters can commonly drop 4 points. This is why quahaugs are good to eat throughout the season, and oysters are watery and unappetising after spawning. The selection of 50 mm clams for the condition indices was in order to reduce variability even though, according Rainer and Mann (1992), there was no significant difference in the indices of clams ranging from 36 mm to 96 mm.

As a whole, the three seasonal studies had a high degree of similarity. For both the G-S ratio and the ovocytes, there was a maximum in the beginning of July and a minimum at the end of September. The condition index suggests a spawning event from late June until August. Eversole (1997), through capturing quahaug larvae in South Carolina, found that spawning

continued throughout the summer. The most likely explanation is that the maxima represent the beginning of spawning, when the gonad is full and eggs are at their largest and most viable. Afterwards, spawning continues gradually throughout the season. The idea of gradual spawning comes from the weak changes in the condition index. This corresponds to the predictions by certain researchers, based on similar condition index studies and an often weak concentration of larvae in the water, that quahaugs "trickle spawn ". As well, Eversole *et al.* (1980), found that quahaugs had a long spawning period. This contrasts strongly with what is seen in spawning oysters that have a large and rapid decrease in the condition index and a high concentration of larvae in the water at that time.

Applications

The information gathered in this study adds to the general knowledge of the quahaug, but it can also be put to practical use in stock management. First of all, one method for improving stocks would be to have closed fishing periods after spawning. Sephton and Landry (1992) demonstrated that any activity on the substrate (i.e. digging or walking) had a negative effect on spat survival. Planktonic larval stages last approximately two weeks (Rice, 1994). A short closed period, two weeks after spawning, could help increase the number of clams on a fisherman's lease. The results of this project indicate that this period could be around mid-July. This would be mainly directed at recreational fishers who tend to be ineffective fishers and spend a lot of time trampling the sediment.

Another possibility for enhancement would be to establish maximum size limit of about 80 mm. Fishermen tend to be less interested in these large clams ("chowders ") because they have a low economic value compared to smaller ones. A "chowder " of >80 mm is worth perhaps $0.10 while a "cherry-stone " of 40-50 mm is often worth $0.35. However, large clams can interfere with recruitment. Larvae trying to settle near a large quahaug can be filtered by it and eaten or trapped in its pseudofaeces (Ahn *et al.*, 1993). Some fishermen will take these large clams and leave them on the beach to die in order to allow more space and less competition for the smaller more profitable ones (Walker and Heffernan, 1996).

Nonetheless, these large clams exhibit a high reproductive potential. This study showed that relative contribution and egg size increased with clam size, meaning larger clams would

probably produce many more abundant and better quality gametes. Peterson (1986) found no sign of reproductive senility in quahaugs, which indicates that clams keep producing well into old age. Based on the relative contribution, one 75 mm female is equivalent to approximately 10 females measuring 40mm. In economic terms, this translates into a value of $3.50 in small clams to have the same production as $0.10 worth of large clams.

The reproductive value of the large clams is clear, but the competition problem remains to be resolved. One easily implemented option would be to create subtidal sanctuaries. This would consist of establishing a refuge area for the larger clams in subtidal waters. This study demonstrated little difference between the two plots (Figure 2) with clams in the subtidal zone tending to spawn slightly earlier than clams in the intertidal. Walker and Heffernan (1990, 1994) showed that growth even increased with the amount of time quahaugs spend submerged. Therefore, rather than removing or killing the large clams, fishermen could place them in deeper water. This way it would be possible to preserve the reproductive biomass while reducing competition. Another advantage of this strategy is that clam growers rarely use the subtidal waters, so these sanctuaries would not decrease the amount of the usable claming areas.

Conclusion

The present work supplied information on the reproductive biology of the quahaug in PEI. The results concerning quahaug biology were similar to research that has been done in the southern United States. However, the timing and method of spawning was different. The three stock enhancement strategies discussed can be applied to quahaug fisheries and aquaculture management. These strategies should now be tested in order to determine their impact. In addition, more research is needed towards a better understanding of recruitment and survival at the larval level and how density affects fecundity.

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Table 1: Sampled size classes.

Classes	Small "littlenecks"	Medium "cherrystones"		Large "chowders"
Size (mm)	<40	40-50	50-60	>60

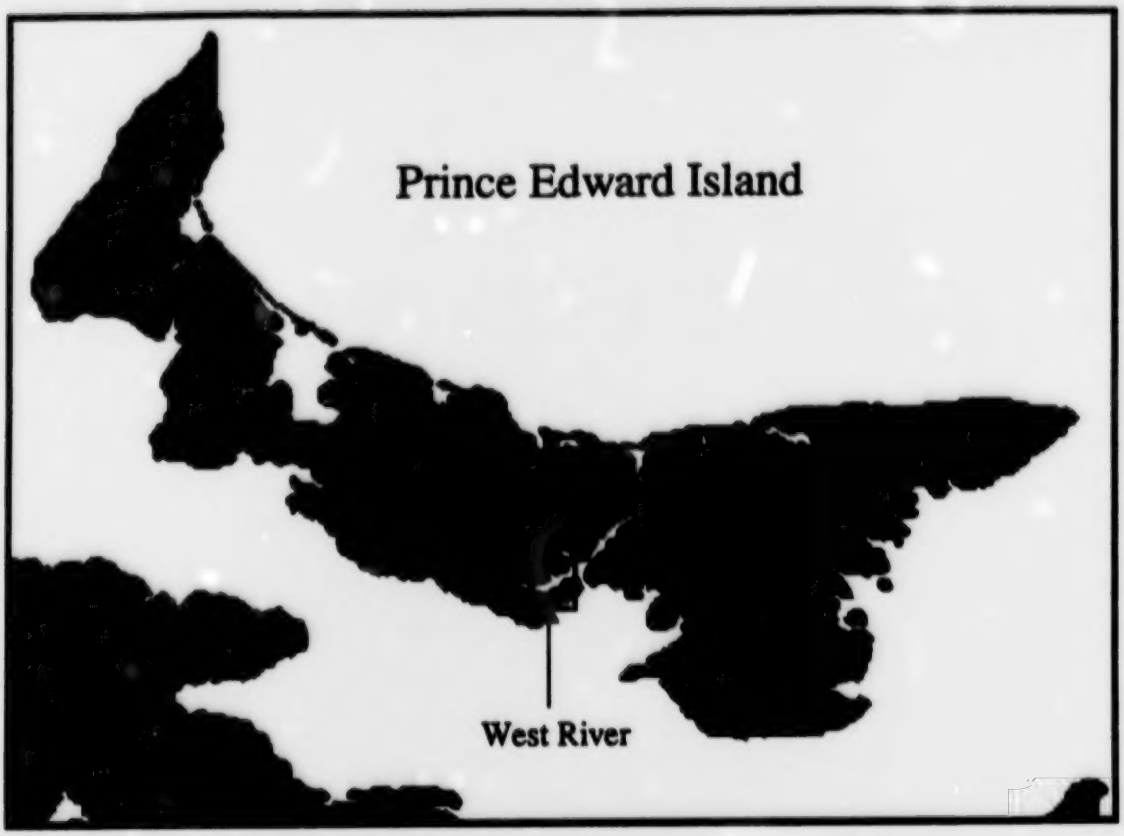

Figure 1 : Location of West River, Prince Edward Island

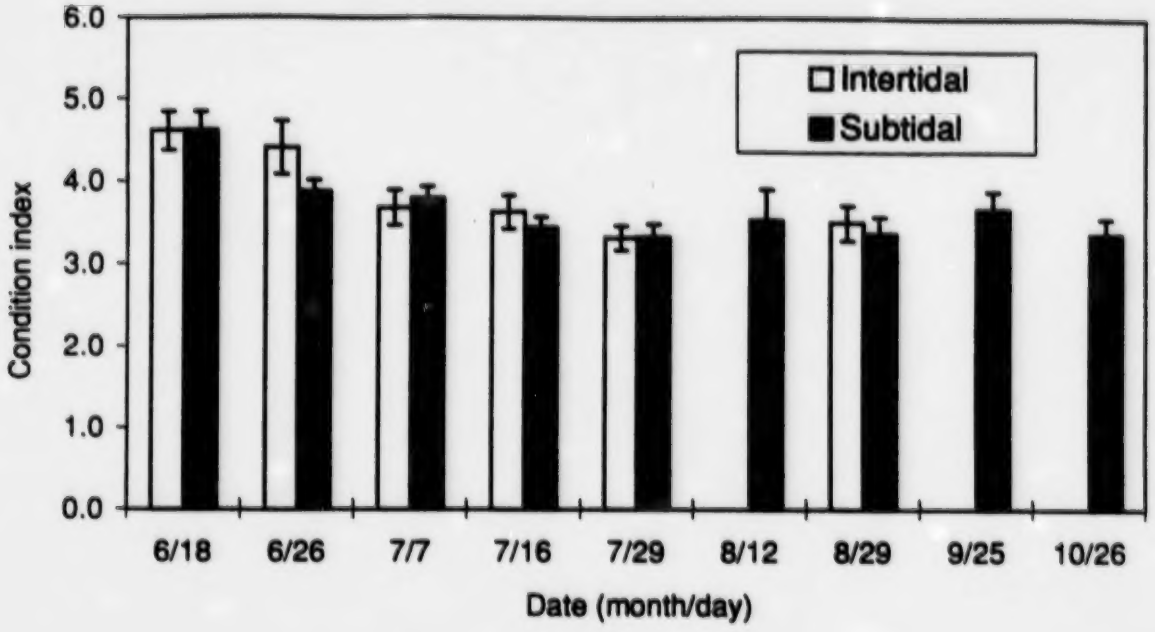

Figure 2: Seasonal size condition indices.

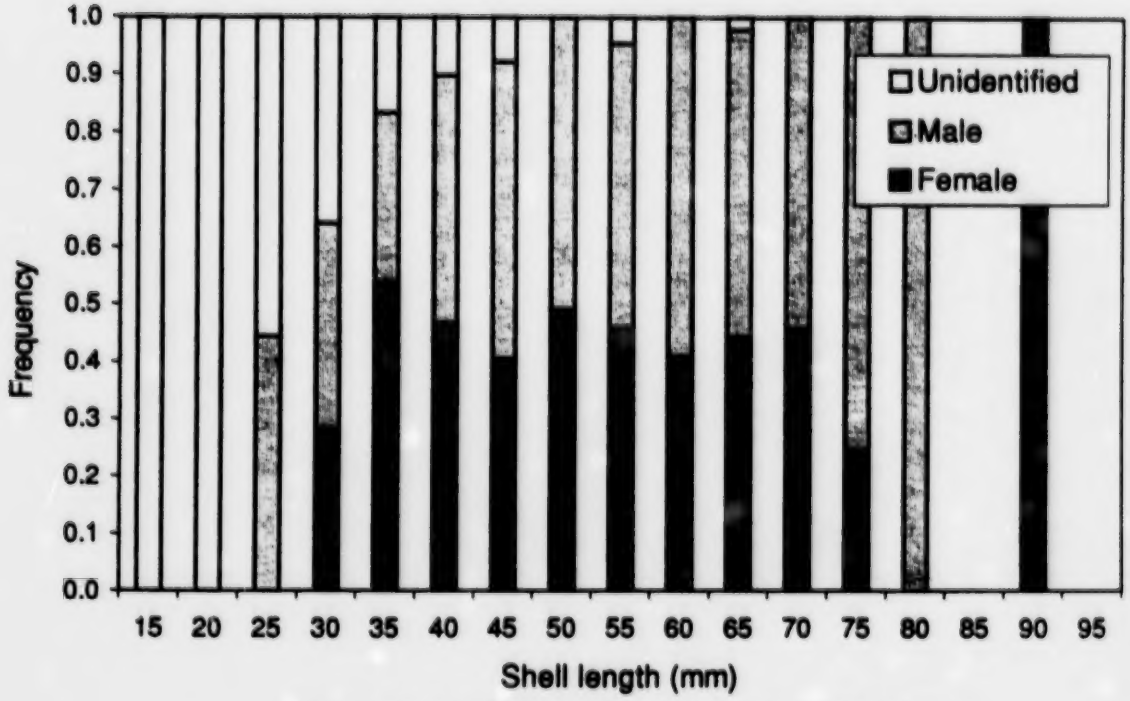

Figure 3: Sex frequencies as a function of size.

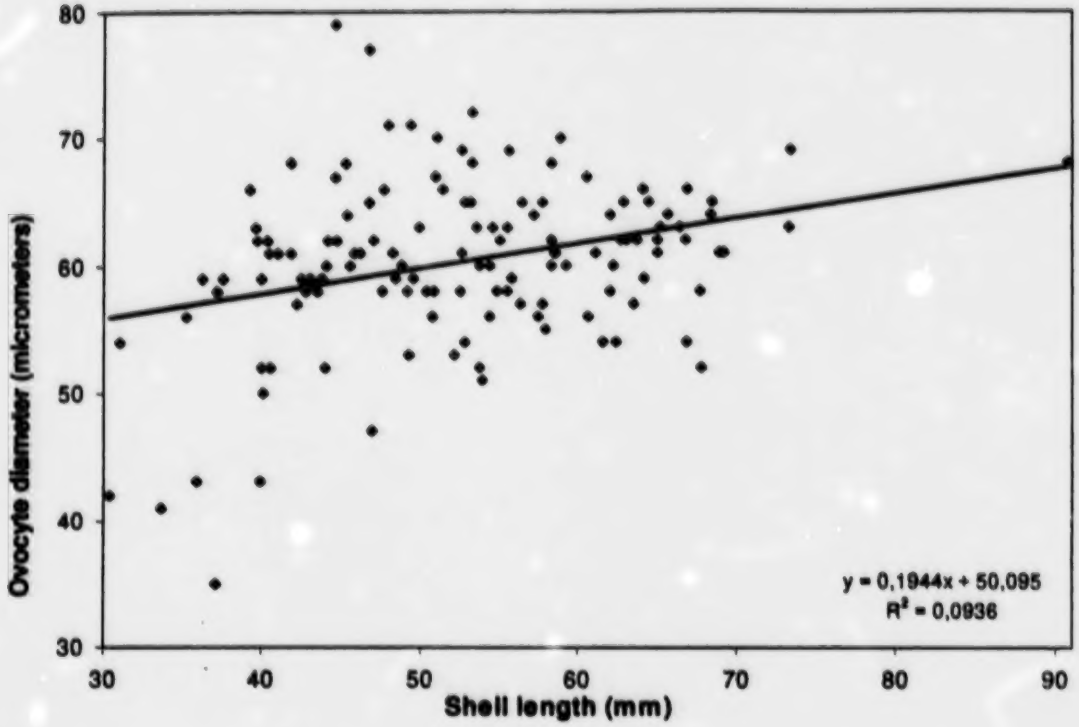

Figure 4: Ovocytes as a function of size.

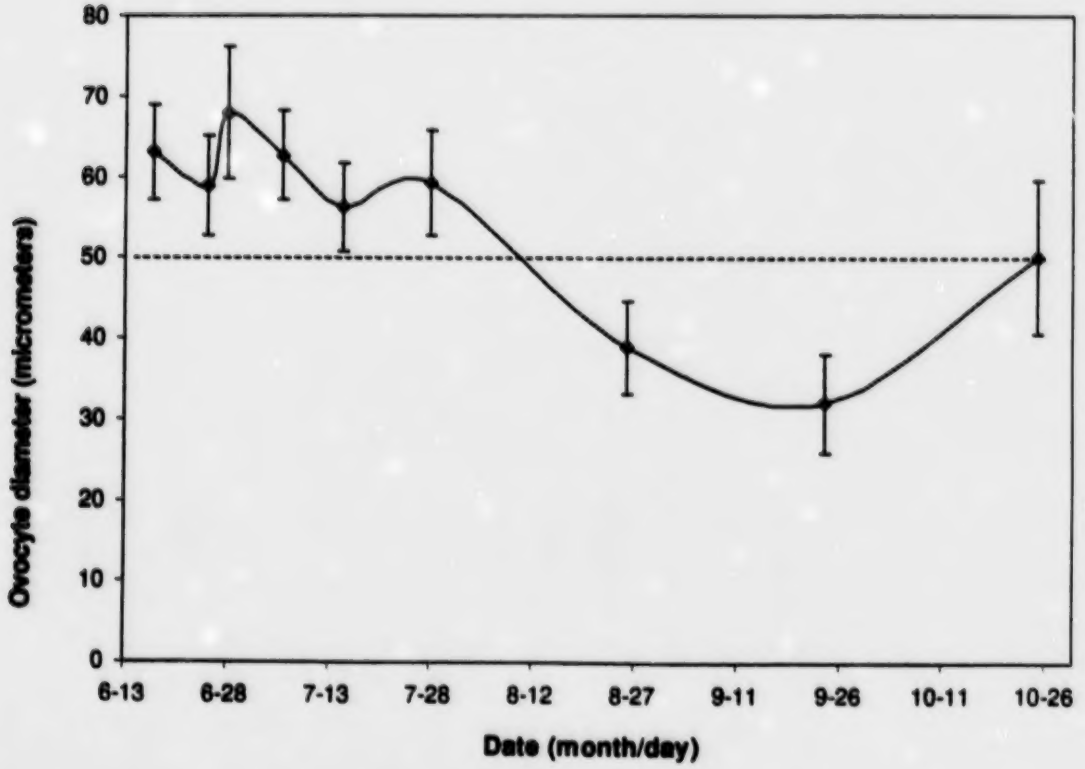

Figure 5: Seasonal ovocyte sizes.

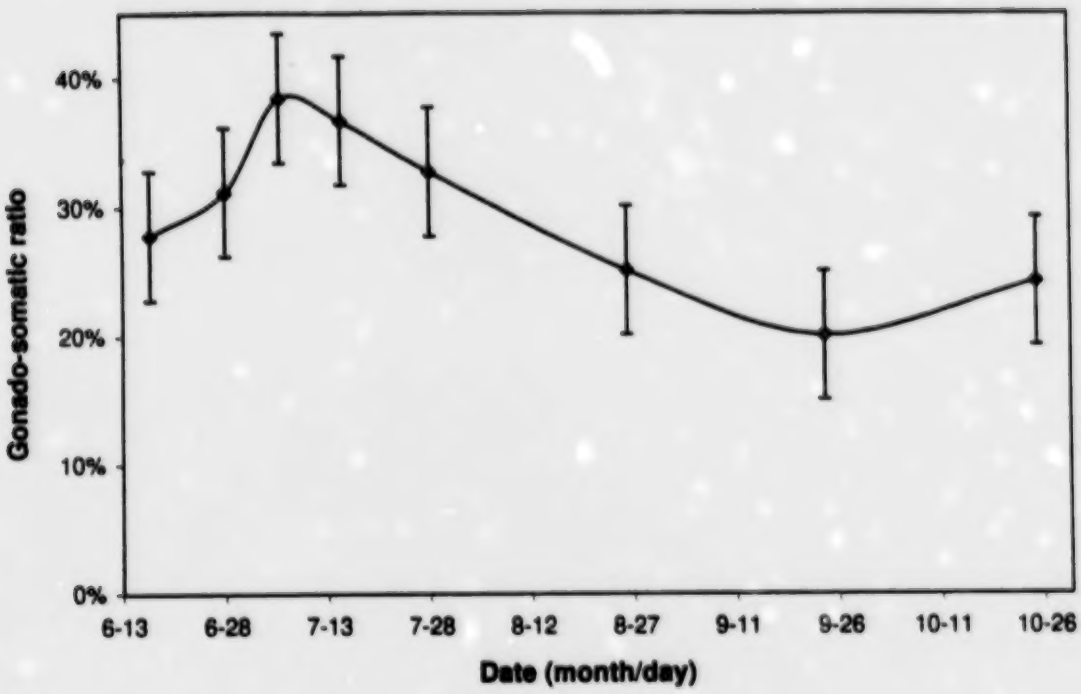

Figure 6: Seasonal gonado-somatic ratios.

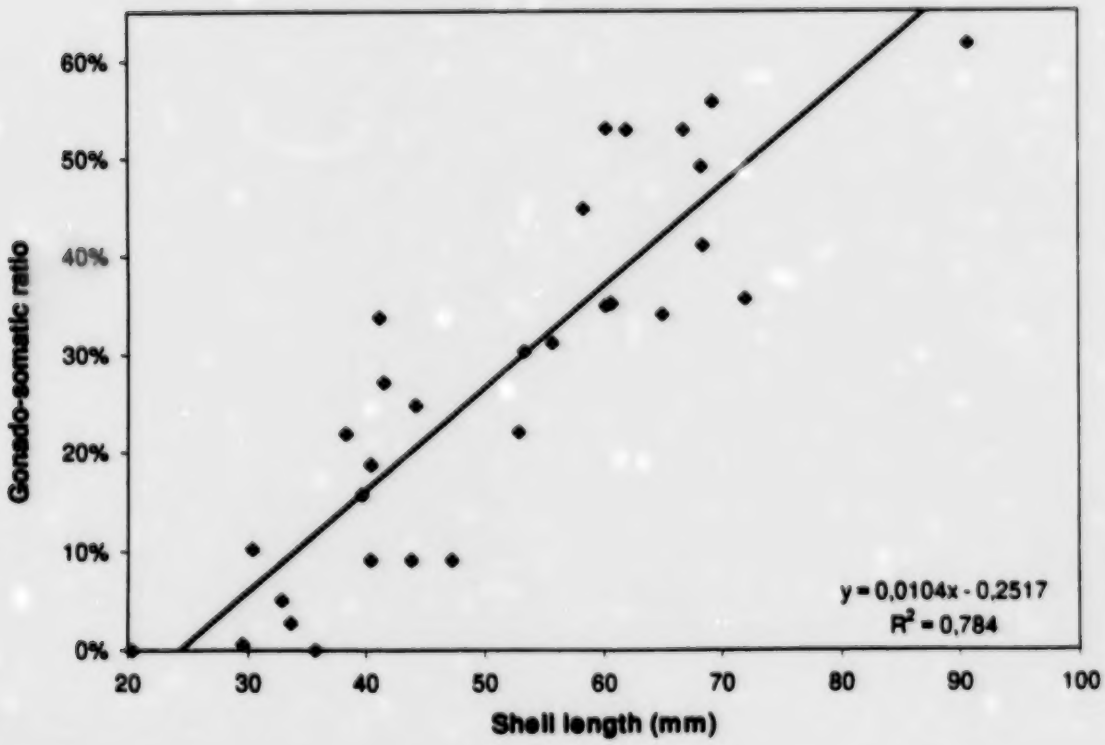

Figure 7: Gonado-somatic ratio as a function of size.